REFLEX CARDIAC INHIBITION OF BRANCHIO-VASCULAR ORIGIN IN THE ELASMOBRANCH, SQUALUS ACANTHIAS

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Reflex cardiac and respiratory inhibition has been observed in elasmobranchs upon mechanical or electrical stimulation of various regions, external and internal, including the gill region (Lutz, 1930*a*). Reflex cardiac inhibition in mammals may also be obtained upon stimulating various regions. The distribution of the latter sensory areas is, however, much more restricted than in the elasmobranchs. Receptors which are especially important in regulating the circulation appear to be located in the vascular organs themselves. The depressor mechanism with receptors located in the aorta is well known (Eyster and Hooker, 1908; Anrep and Segall, 1926), and recently the carotid sinus has been shown to be an important zone, reflexly controlling heart rate and vasomotor tonus (Heymans, 1929). The physiological stimulus is apparently an alteration of pressure within this vessel.

The present paper is concerned with an attempt to ascertain whether similar alterations of pressure within the gill vessels of elasmobranchs are effective cardio-inhibitory stimuli.

Material and Method

Specimens of the dogfish, *Squalus acanthias*, 600 to 1500 grams in weight, taken during the month of August from Frenchman Bay, Maine, were used. The spinal cord was pithed posteriorly from the level of the sixth vertebra. The fish was secured ventral side up in a shallow tank of sea water. Although respiration continued in an apparently normal way, perfusion of the gills with sea water through the mouth was carried out in most cases, in order to insure an adequate supply of oxygen. The ventral aorta was exposed and ligated between its first and second branches. The former give rise to the first and second afferent branchial arteries which supply the hemibranch and the first holobranch. Therefore the second, third and fourth holobranchs were functional. Changes of pressure in the first branches and their derivatives were effected through a cannula inserted in the ventral aorta, anterior to the ligature, and connected with a burette filled with a physiological solution (urea-saline, Lutz, 1930*b*). The heart beat was recorded on a smoked drum by means of a heart lever or a mercury manometer recording ventral aortic pressure through a cannula inserted posteriorly to the ligature.

Results

Cardiac inhibition was obtained when the first ventral aortic branches, and thus the gill vessels arising from them, were suddenly subjected to increases of internal pressure applied by opening the connection between the cannula and the burette for varying periods (Fig. 1, A). The amount of fluid entering during the application of pressure varied from 0.2 cc. to 5 cc., depending upon the height at which the burette was set and the length of time it was held open.

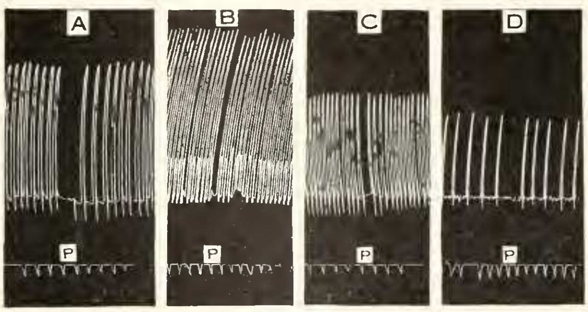

FIG. 1. Reflex cardiac inhibition following sudden increase of pressure within the gill vessels at *P*. Heart beat recorded by lever. In this and in subsequent figures the time record shows five-second intervals. Cord pithed posteriorly from the level of the sixth vertebra. Urea-saline solution in pressure burette except in B. A. Increase of pressure in the first branches of the ventral aorta. B. Same, with dogfish blood in the pressure burette. C. Same, with gill capillaries occluded with cornstarch so that no fluid entered. D. Increase of pressure in the third, fourth and fifth branchial arteries.

Inhibition was invariably obtained when the pressure applied (50 to 125 cm. of urea-saline) was obviously much higher than the dorsal aortic blood pressure. Simultaneous measurements of the ventral and dorsal aortic blood pressures showed that the pressure differences between the two aortae averaged 16.1 mm. Hg for systolic pressure and 4.3 mm. Hg for diastolic pressure, and that the pulse wave is transmitted through the gill capillaries to the dorsal aorta (Fig. 2, A). It is apparent, therefore, that the gill capillaries are relatively wide, and that the pressure in the afferent aortic system beyond the ligature is essentially the same as that in the dorsal aorta. Dorsal aortic systolic pressure ranged from 11 to 28 mm. Hg (8 measurements), and it was noted that fluid would not enter the ventral aorta from the burette below 25 cm. urea-saline (18.6 mm. Hg). A sudden increase of pressure averaging 10.7 mm. Hg above the average systolic pressure existing in the dorsal aorta was found to constitute an adequate stimulus for cardiac inhibition (Fig. 2, B), and in one instance a difference as low as 3 mm. Hg produced the response (Fig. 2, C).

Although urea-saline is considered to be a physiological solution, a control experiment was performed using heparinized fresh dogfish blood in the burette and its connections. A similar inhibitory response to increased pressure in the gill vessels was obtained (Fig. 1, B).

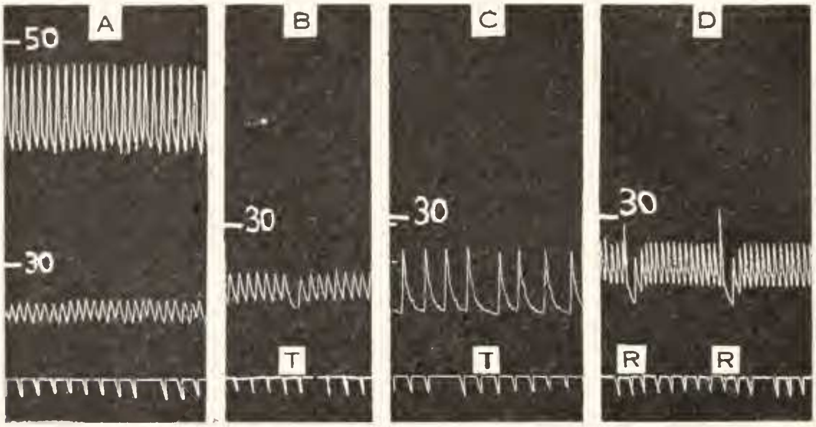

FIG. 2. Heart beat recorded by Hg manometer. Figures at left show pressure levels in mm. Hg. A. Simultaneous records of ventral (upper) and dorsal (lower) aortic blood pressure. B. Ventral aortic blood pressure record. Threshold increase of pressure in the first branches of the ventral aorta at *T* (33 cm. urea-saline, 24.6 mm. Hg). C. Same, threshold 25 cm. urea-saline (18.6 mm. Hg). D. Ventral aortic blood pressure record. Spontaneous ejection reflexes at *R*.

Cardiac inhibition was obtained when the burette was opened and immediately closed, allowing only 0.2 cc. of fluid to enter. Furthermore, holding the burette open, until 5 cc. of fluid had entered the ventral aorta, did not increase or continue the initial inhibition during the period of flow. It follows that the effective stimulus is the initial increase of pressure *per se*, and not flow of fluid through the gill vessels. This was substantiated by blocking the flow through the gill capillaries so that the application of pressure alone served to stimulate. Attempts to do this by tying the efferent branchial supply from the hemibranch and first holobranch were unsuccessful because of the numerous anastomoses between these vessels and the neighboring efferent system. An effective block on the afferent side was obtained by occluding the gill capillaries with cornstarch, injected in suspension in urea-saline solution, through the ventral aorta. This suspension was withdrawn from the larger afferent vessels with a syringe and the vessels were refilled with urea-saline, thus giving a vascular pocket on the afferent side against which pressure could be applied without passage of fluid (Fig. 1, C).

That the receptors for the cardio-inhibitory response to increase of pressure within the gill vessels are not limited to the first two branchial arteries was shown by an experiment in which the ventral aorta was ligated between its first and second branches, and pressure applied to the third, fourth and fifth branchial arteries through a cannula inserted through the conus arteriosus (Fig. 1, D).

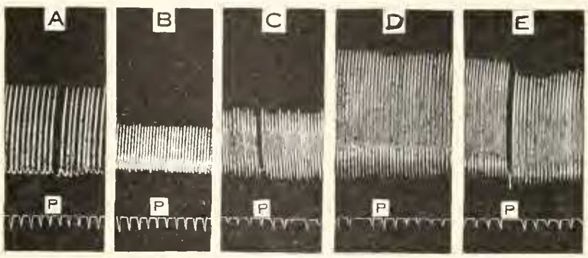

FIG. 3. Sudden increase of pressure within the first branches of the ventral aorta at *P*. Heart beat recorded by lever. A, before and B, after cutting the vagus supply to the heart on both sides. C. Vagus supply to heart cut on right side and increase of pressure in right gill vessels (crossed reflex). D, increase of pressure in right gill vessels, and E, in left gill vessels after cutting cranial nerves from fifth through tenth on right side (afferent supply from gills).

The cardiac inhibition following increase of pressure within the gill vessels is a reflex response inasmuch as it disappeared when the brain was destroyed by pithing. When the ramus posttrematicus of the fourth branchial division of the vagus (5th branchial nerve) and the ramus visceralis were cut on both sides the reflex was also abolished, thus showing that the efferent side of the reflex is in the vagus supply to the heart (Fig. 3, A, B). When these nerves were cut on the right side and the first right branch of the ventral aorta was clamped, thus leaving only the left gill vessels open to stimulation, the usual inhibition was obtained. When the clamp was shifted to the first left branch of the ventral aorta, the response was also obtained upon stimulating the right gill vessels (Fig. 3, C). Evidently the pathways for this reflex are both unilateral and crossed in the central nervous system. The threshold of pressure for the crossed reflex was about 10 cm. of urea-saline higher than that for the reflex obtained from stimulation of both branches.

The afferent innervation of the hemibranch and the first two gills was interrupted on both sides by cutting the first three branchial nerves (ninth and the first and second branchial divisions of the vagus). Following this no cardiac response to increase of pressure in both first branches of the ventral aorta was obtained. The integrity of the remaining cardio-inhibitory reflex mechanism was demonstrated by obtaining reflex inhibition upon pinching with forceps the skin between the fourth and fifth gill slits, the snout, the base of the pectoral fin, or upon cutting open the abdomen or scratching the wall of the pericardial chamber with a needle. The afferent side of the reflex following increase of pressure within the anterior gill vessels is thus located.

Inasmuch as cutting the first three branchial nerves involves opening the anterior cardinal sinus with considerable hemorrhage, the afferent nerve supply from the gills was interrupted on one side by transecting the cranial nerves from the fifth through the tenth close to the brain with a single incision through the skin. Following this, the usual cardio-inhibitory reflex was not obtained from the gill vessels on the operated side. Mechanical stimulation of the gill region on this side also failed to elicit a reflex, but both increase of internal vascular pressure and mechanical stimulation of the gills on the intact side evoked the cardio-inhibitory response (Fig. 3, D, E).

Discussion

The blood pressure in the elasmobranch has been found to be of the low pressure type. Thus Schoenlein (1895) found in *Torpedo* a branchial systolic pressure of 16 to 18 mm. Hg and in *Scyllium* one of 30 to 33 mm. Hg; Hyde (1908) found the mean pressure in a branch of the aorta of the skate to be 20 mm. Hg; Lyon (1926) found in sand sharks an average branchial pressure of 32 mm. Hg and an average dorsal or systemic pressure of 23.3 mm. Hg. In *Squalus acanthias* we found the average pressure in the ventral aorta to be 28.2 mm. Hg and that in the dorsal aorta to be 15.4 mm. Hg. The average pulse pressure in the ventral aorta, as recorded by the mercury manometer, was 13.3 mm. Hg which is 47.2 per cent of the average systolic pressure. The ventricular beat as a factor in maintaining blood pressure is obviously important. This is in accordance with the fact that no vasomotor innervation has been demonstrated in these fishes. The heart reflexes, therefore, must be of importance as regulatory factors for the vascular needs of the body. The cardio-inhibitory reflex, as shown above, can be elicited by an increase in gill blood pressure well within physiological limits, and may temporarily decrease the diastolic blood pressure to a significant degree. In one case this decrease was 4 mm. Hg, which was 50 per cent of the diastolic pressure. Such a mechanism might come into play during an ejection reflex, when the sudden constriction of the branchial muscles forces water from the pharynx. The accompanying external pressure on the gill vessels would force blood from them in both directions. The blood pressure in the ventral aorta would rise, inasmuch as the blood would meet the valves in the conus arteriosus, evoking a sudden need for cardiac inhibition to prevent dangerous consequences, such as injury to the thin-walled afferent system. The increase in internal pressure would evoke the reflex, momentarily preventing additional pressure increase due to the ventricular action. As a matter of fact, our records show an increase of ventral aortic blood pressure well above threshold value followed by cardiac inhibition during spontaneous ejection reflexes (Fig. 2, D). This mechanism, therefore, being of physiological significance, may be compared with the carotid sinus mechanism in mammals. Cardiac inhibition during an ejection reflex may also be evoked by the mechanical stimulation of the gill region, inasmuch as the receptors for the heart reflexes have a wide distribution in the elasmobranch.

It is conceivable that in the course of evolution the widespread sensitive areas of the ancestral form, possibly typified by the elasmobranch, were concentrated or delimited until the condition seen in the mammal was reached. The carotid arteries of the mammal are derivatives of the primitive branchial system. The cardio-inhibitory reflex following increase of pressure within the gill vessels in elasmobranchs may exemplify, therefore, the evolutionary forerunner of the carotid sinus mechanism of mammals as it existed in whatever may have been the ancestral form. This is one of many instances in which it is apparent that physiological as well as morphological factors should be considered in evolutionary reasoning.

Summary

1. Cardiac inhibition follows sudden increase of pressure within the gill blood vessels of *Squalus acanthias*. An average increase of 10.7 mm. Hg above the average systolic pressure existing in the dorsal aorta constitutes an adequate stimulus for the inhibitory response.

Respiratory reflexes were frequently seen associated with the cardio-inhibitory reflex induced by changes in blood pressure. Inasmuch as the carotid-sinus mechanism in mammals has recently been shown to be concerned in the regulation of respiration, further work on the peripheral control of respiration through receptors within the gill vessels of elasmobranchs is in progress.

2. The cardio-inhibitory response is a reflex, with afferent pathways located in the branchial nerves and efferent pathways in the vagus supply to the heart. The reflex is both unilateral and crossed.

3. The average ventral aortic systolic blood pressure in *Squalus acanthias* is found to be 28.2 mm. Hg, the average dorsal aortic systolic pressure 15.4 mm. Hg, and the average ventral aortic pulse pressure 13.3 mm. Hg. The inhibitory reflex to increased ventral aortic pressure modifies the diastolic blood pressure to a significant degree.

4. The adaptive nature of the reflex is pointed out and its phylogenetic significance is discussed.

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